ELECTRONICALLY FILED ON: **MARCH 25, 2009**		
DECLARATION OF YADONG HUANG UNDER 37 C.F.R. § 1.132	Attorney Docket Confirmation No.	GLAD-281 3423
	First Named Inventor	Yadong Huang
	Application Number	10/627,447
	Filing Date	July 24, 2003
	Group Art Unit	1641
	Examiner Name	Ann Y. Lam
Address to: Commissioner for Patents Alexandria, VA 22313-1450	Title	*Method of diagnosing Alzheimer's Disease*

Dear Sir:

1. I, Yadong Huang, declare and say I am the inventor of the claims of the above-identified patent application. I directed others and personally performed the research leading to the invention disclosed and claimed therein.

2. I have read the Office Action dated July 24, 2008 and the Advisory Action dated January 16, 2009 in this application. I understand that the Examiner has rejected pending claims 1-8, 10-14, 19, and 20, and that the Office Action has asserted that these claims are obvious in view of Roses et al. (U.S. Patent No. 5,508,167; "Roses") in view of Huang et al. ((2001) *Proc. Natl. Acad. Sci. USA* 98:8838; "the Huang reference").

3. As discussed during the telephone interview that took place on March 4, 2009, the Huang reference is from my own work. Also as discussed during the March 4, 2009 telephone interview, it would not have been obvious to those in the field, as of the July 30, 2002 priority date of this application, that carboxyl-terminal truncated apoE would be found in the biological fluids such as serum and cerebrospinal fluid (CSF). These points are elaborated upon in the following paragraphs.

The Huang Reference

4. The Huang reference is from my own work. I am the first author on the Huang reference, and directed my co-authors on that paper. In the Huang reference, we reported that carboxyl-terminal truncated apoE is present in brains of AD patients. Huang reference, page 8839, column 2, first paragraph under "Results." We also reported that carboxyl-terminal truncated apoE was detected in the lysates (not in the culture medium) of transfected Neuro-2a cells expressing apoE3 or apoE4. Huang reference, page 8840, column 1, first paragraph. Finally, we reported that carboxyl-terminal truncated apoE is present in intracellular inclusions.

5. Based on the observations reported in the Huang reference, I did not believe that carboxyl-terminal truncated apoE would be found in aqueous biological samples such as plasma, serum, or CSF.

6. Indeed, work from my laboratory subsequently showed that production of carboxyl terminal-truncated apoE in the body is **neuron specific**. Brecht et al. ((2004) *J. Neurosci.* 24:2527; "Brecht"). I am the senior author on Brecht. Brecht notes that apoE proteolysis, resulting in carboxyl-terminal truncated apoE, is neuron specific. This observation, and in view of the observation in the Huang reference that carboxyl-terminal truncated apoE is in insoluble, intracellular formations, would not have led those in the field to expect that carboxyl-terminal truncated apoE would be found in the serum, plasma, or other aqueous biological sample.

It has been reported that apoE synthesized in the brain does not enter the peripheral circulation.

7. Apolipoprotein E (apoE) is synthesized in the brain and by other organs such as the liver. About 90% of serum apoE is synthesized by the liver. Studies have shown that **apoE synthesized by the liver does not enter the brain from the plasma**; and that **apoE synthesized in the brain does not enter the plasma**. See, e.g., Linton et al. (1991) *J. Clin. Invest.* 88:270 ("Linton 1991"). As such, as of the July 30, 2002 priority date of this application, those in the field would not have expected apoE synthesized in the brain to be present in plasma.

8. Linton describes the apoE phenotypes present in serum of human liver transplant recipients. Table I of Linton 1991 shows that the serum apoE phenotype of liver transplant recipients **is the same as that of the donor liver, and not of the recipient**. If apoE synthesized in the brain were to enter into the peripheral circulation, one would expect that the apoE phenotype in the serum of liver transplant recipients would be a

mixture of the recipient's preoperative serum apoE phenotype and the donor apoE phenotype. It is not. The source of the apoE in the liver transplant recipient is the donor liver; hence, the serum apoE phenotype of the liver transplant recipients is the same as that of the donor liver. This study shows that **apoE synthesized in the brain does not enter the plasma**.

Serum apoE is synthesized by the liver, and by macrophages.

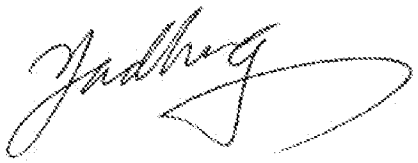
9. As noted above, about 90% of serum apoE is synthesized by the liver. The other ~10% of peripheral apoE is synthesized by macrophages, as reported in a bone marrow transplantation study by Linton et al. ((2005) *Science* 267:1034; "Linton 2005"). Linton 2005 describe transplantation of $apoE^{-/-}$ mice with bone marrow from $apoE^{+/+}$ or $apoE^{-/+}$ mice. Linton 2005 states that after transplantation, serum apoE levels in $apoE^{-/+} \rightarrow apoE^{-/-}$ mice were about 5.5% of normal, and that serum apoE levels in $apoE^{+/+} \rightarrow apoE^{-/-}$ mice were about 12.5% of normal. See Linton 2005, first paragraph on page 1035. These results indicate that peripheral macrophages synthesize about 10% of peripheral apoE. These results have been confirmed by others in the field.

Detection of carboxyl-terminal truncated apoE in an aqueous biological sample would not have been obvious in view of Roses.

10. Roses discusses detecting apoE4 as a means of identifying individuals at risk of developing Alzheimer's Disease. However, it has been reported in many studies that the predictive values of apoE4 genotyping do not support its utility as a diagnostic test for Alzheimer's disease. In a conference that held in Chicago, Illinois, in October 1995, a working group of the National Institute on Aging (NIA) and the Alzheimer's Association drafted consensus recommendations on research and clinical applications of apoE4 testing for Alzheimer's disease (AD). Their conclusions were reported in Relkin et al. ((1996) *Ann. NY. Acad. Sci.* 1802:149; "Relkin"). Relkin states that the working group "recommended against the use of APOE genotyping to predict the future development of AD in asymptomatic individuals at this time, and warned against the use of the test in isolation as the sole means for diagnosing AD." Relkin, page 162, Summary.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment,

or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



March 25, 2009

Date

Yadong Huang

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